BOUND WATER IN *ASPERGILLUS NIGER*

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(WITH TWO FIGURES)

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The concept of bound water has been defined and explained in many ways (**2**). It is not the purpose of the present investigation to extend the controversy, but simply to find out whether physiological changes produced in plants as a result of exposure to specific environmental vapor pressures can be explained in terms of a binding of water by (or hydration of) specific substances in the plant.

Evidence has repeatedly been produced both for and against a relationship between bound water and both frost and drought resistance (**2, 4, 5**). Two main difficulties have prevented the resolution of this point: (a) the use of inadequate methods that may be measuring something other than bound water (**3**) and (b) the use of higher plants whose cells have large vacuoles capable of storing high concentrations of sugars and other non-protoplasmic substances that may conceivably bind water. Any binding of water by protoplasmic substances under these circumstances would be difficult to detect.

In the present investigation these difficulties have been overcome (a) by using a very simple but accurate method of measuring bound water, (b) by using a microorganism whose cells contain very little vacuolar material and therefore consist very largely of protoplasmic substances. The organism is also capable of growth in a wide range of vapor pressures.

Methods

A. CULTURAL.—Flasks containing the necessary quantity of Steinberg's dibasal medium (50 ml. in 125-ml. flasks or 200 ml. in one-liter flasks) were autoclaved at 15 pounds pressure for 20 minutes. The cooled media were inoculated with spores from a pure culture of *Aspergillus niger*. The mycelium was allowed to grow for about seven days at 25° C, before harvesting. The osmotic pressure of the solutions was controlled by adding the necessary amounts of dextrose (from 50 to 547 g./l.).

B. OSMOTIC PRESSURE DETERMINATIONS.—Two methods were used to determine the osmotic pressures of the culture media: (1) by measuring the freezing-point lowerings of the solution, using either a Beckmann thermometer or a copper-constantin thermocouple connected to a Leeds and Northrup Type K potentiometer and a galvanometer; (2) by calculation from the concentrations of sugars, using the values given in the International Critical Tables.

C. BOUND WATER DETERMINATIONS.—At the end of the growing period, the mycelial mats were harvested, washed, blotted, then dried in weighing bottles over a desiccant (Al_2O_3 or $Mg(ClO_4)_2$) in an evacuated desiccator at room temperature until equilibrium was attained (one to two months). The weighing bottles were then transferred to an oven at 80° C or 110° C and again dried until equilibrium was reached (one to two months). The difference between these two weights was taken as the bound water, which was expressed as a per cent. of the oven-dry weight. Bound water determined by this method is simply the water held by the mycelium at room temperature in a vacuum but driven off at 80° C or 110° C respectively.

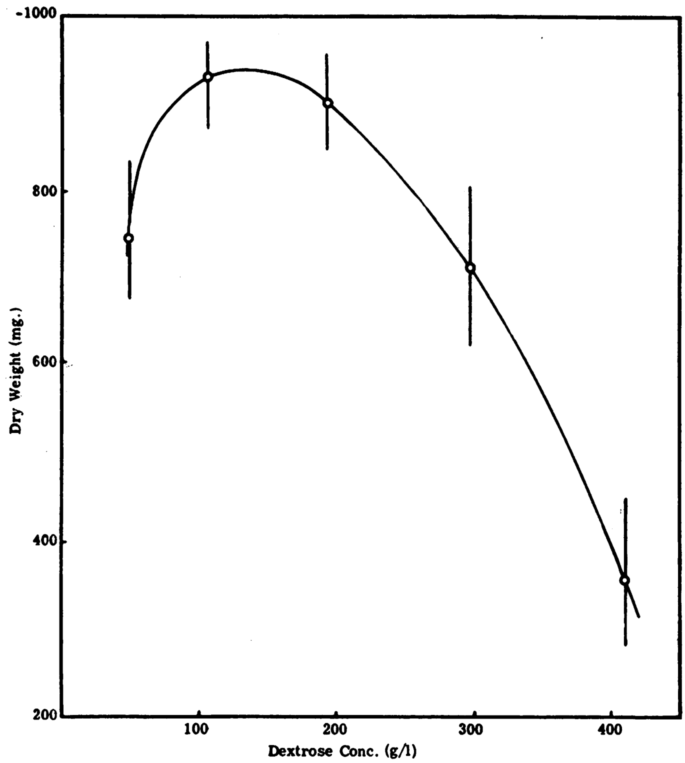

FIG. 1. Growth of *A. niger* in culture media containing various amounts of dextrose. Each point is an average of three determinations, the spread being shown by the straight line through points.

Experimental methods

A. OSMOTIC PRESSURES OF CULTURE SOLUTIONS.—There was good agreement between the values obtained from freezing-point determinations and those calculated from the sugar concentration. The calculated values were always about 10% higher, and are adopted in the following experiments, since they are not open to complicating factors such as undercooling and possible crystallizing out of solute which may occur during freezing-point determinations.

B. Growth of the organism.—Figure 1 shows the actual amounts of growth obtained at the different osmotic pressures using dextrose. Microscopic observations showed that the cells were much smaller in the solutions of higher osmotic pressures. A few small vacuoles were observed in each cell when grown at the lowest osmotic pressure, but none could be detected in the cells grown at the higher osmotic pressures.

C. Bound water of fungus mycelium grown at different osmotic pressures.—Table I shows the results obtained with cultures grown in triplicate at six different osmotic pressures and dried over Al_2O_3. Although

TABLE I

BOUND WATER OF *A. niger* MYCELIUM IN RELATION TO OSMOTIC PRESSURE

O.P. of medium (in atm.)	Sporulation*	pH of medium at harvest**	Dry weight at 110° C (in mg.)	Bound water content at room temperature (in mg.)	Bound water %
7.7	1	2.6	396.4	31.4	7.9
7.7	3	2.5	290.8	24.3	8.4
7.7	3	2.7	286.2	20.2	7.1
15.5	3	2.6	420.8	28.2	6.7
15.5	3	2.5	397.9	25.4	6.4
15.5	5	2.8	413.4	30.4	7.4
30.6	5	2.4	470.5	38.0	8.1
30.6	4	2.4	409.8	35.3	8.6
30.6	4	2.6	327.8	20.9	6.4
52.7	2	2.3	442.0	78.8	17.8
52.7	1	2.4	250.7	42.3	16.8
52.7	3	2.5	370.6	28.6	7.6
83.7	0	2.2	371.1	120.4	32.4
83.7	0	2.5	64.6	15.8	24.5
83.7	0	2.4	184.1	41.5	22.5
132.2	0	2.1	273.3	73.1	26.7
132.2	0	2.1	260.4	74.2	28.5
132.2	0	2.1	319.1	97.1	30.4

*Sporulation: 5 = heavy; 0 = none.
**Original pH 4.4–5.6.

the three series of cultures at the lowest osmotic pressures contained about the same amount of bound water, the four grown at the highest osmotic pressures showed a direct relation between osmotic pressure and bound water.

In the next experiment $Mg(ClO_4)_2$ was used instead of Al_2O_3 and since the former maintains a lower vapor pressure (1), one would expect lower bound water values, and this is exactly what was obtained (fig. 2). Furthermore the bound water is greater when the mycelium is dried at 110° C than when it is dried at 80° C. But in these two cases the correlation is even better. Only the culture at the highest osmotic pressure shows a bound water value (average 16.58) that is out of place. This series of cultures, however, had to be grown twice as long as the others in order to obtain enough mycelium and is therefore not strictly comparable.

There is thus no doubt that the water bound by the mycelium of *Aspergillus niger* increases with an increase in the osmotic pressure of the medium in which it is grown.

In the case of higher plants (**5**) such correlations can be easily explained

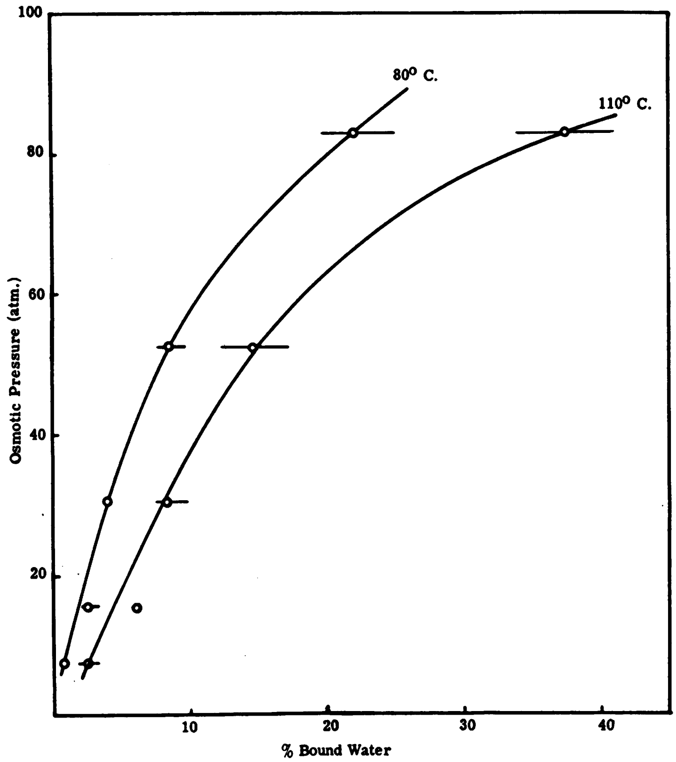

FIG. 2. The relationship between per cent. bound water of *A. niger* mycelium and osmotic pressure. Each point is an average of three determinations, the spread being shown by the straight line through points.

$$\% \text{ Bound Water} = \frac{\text{d.w. at room temp.} - \text{d.w. at } 80^\circ \text{ C (or } 110^\circ \text{ C)}}{\text{d.w. at } 80^\circ \text{ C (or } 110^\circ \text{ C)}} \times 100$$

by the increased sugars (**4**). It is therefore important to investigate this possibility in *A. niger*. Although the mycelium was well washed before drying, it is conceivable that small amounts of sugar remained in the case of the mats grown at higher concentrations. Furthermore, in spite of the paucity of vacuoles, the cells may conceivably accumulate some sugar. It should be pointed out, however, that in order for sugars to account for the high bound water of the mycelium grown at high osmotic pressures, the sugar itself would have to bind even larger amounts, since the mycelial values would be the averages between low-binding non-sugar and high-binding sugar molecules.

In order to investigate this possibility, aliquots of culture medium were treated in the same way as the mycelium. Table II shows the results. The

TABLE II

BOUND WATER OF THE CULTURE MEDIUM USED FOR OSMOTIC PRESSURE STUDIES

O.P. of medium (in atm.)	Sugar concentration of medium (g/l.)	Dry weight at 80° C (in mg.)	Bound water content at room temperature (in mg.)	% Bound water
7.7	50	87.8	10.6	12.1
15.5	106	199.5	17.7	8.9
30.6	193	365.9	10.4	2.8
52.7	297	548.2	14.9	2.7
83.7	410	762.3	16.8	2.2
132.2	547	917.9	6.8	0.7

highest value was much less than the highest value obtained for the mycelium. Furthermore, the relation to osmotic pressure was the reverse of that obtained with the mycelium, *i.e.*, the medium of lowest concentration had the highest bound water. This may possibly be due to the higher proportion of mineral salts or perhaps partly to the greater tendency for the more concentrated sugar solutions to form an impermeable surface crust. In any case, the relation between the water bound by the mycelium and the osmotic pressure of the medium in which it is grown cannot possibly be explained by the sugars.

The only substances that are both capable of binding large amounts of water and are present in the mycelium in sufficient quantity to account for the increase in bound water are the proteins. Further investigations are being made to determine whether or not this point can be proven.

Summary

1. The term bound water is used for water held at room temperature in an evacuated desiccator containing a desiccant, but driven off in an oven at 80° C or 110° C respectively.

2. The quantities of such bound water held by the mycelium of *Aspergillus niger* varied directly with the osmotic pressure of the solution in which it was grown.

3. These differences could not be accounted for by an accumulation of sugars since the sugars bind far less water than the mycelium grown at higher osmotic pressures.

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